The kinetics of inactivation of spheroidal microbial cells by pulsed electric fields

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Abstract. The nature of non-exponential kinetics in microbial cells inactivation by pulsed electric fields (PEF) is discussed. It was demonstrated that possible mechanism of non-exponential kinetics can be related to orientational disorder in suspension of microbial cells of anisotropic form. A numerical studies of spheroidal cell suspensions was carried out. The most pronounced deviations from the exponential kinetics were observed for disordered suspensions of prolate spheroids at small electric field strength E or at large aspect ratio a. For partially oriented suspensions, efficiency of inactivation enhances with increasing of order parameter and field strength. A possibility of the PEF-induced orientational ordering in microbial suspensions is discussed.

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1. Introduction

Application of high intensity pulsed electric field (PEF) as a nonthermal preservation method have been a topic of growing interest over the past decade [1, 2]. PEF processing is a promising non-thermal alternative technology and many investigators have shown it effectiveness for killing bacteria in liquid aqueous media [3, 4, 5, 6]. The application of high electric field microsecond pulses (typically 10-50 kV/cm) allows inactivation of microorganisms and enzymes [7, 8, 9, 10, 11]. However, the mechanism of inactivation are not yet fully understood. The important problem is to elucidate how kinetics and degree of inactivation depend on the type and geometrical parameters of microorganisms, properties of liquid media and their flow regimes, the temperature and treatment protocol (electric field strength E, wave forms, pulse duration t_i, total time of treatment) [12, 13].

The microbicidal effect of PEF treatment is related to selective damage of the biological membrane in microorganism. In an external electric field E, a transmembrane potential u_m is induced on membrane. When transmembrane potential exceeds some threshold value (typically about 0.2-1.0 V), an electric field cause a temporary loss of the semipermeability of cell membranes or their electroporation [14, 15]. The sufficiently strong electric field and long time of PEF treatment leads to complete membrane damage and cell death [16].

The surviving fraction $S(t, E)$, defined as the ratio of the number of undamaged microbial cells to the total number of microbial cells, decreases with PEF treatment time t. Different empirical models, such as Fermi, Hulsheger, log-log and log-logistic, were proposed for description of inactivation kinetics [3, 17, 18, 19]. Although these models are very popular, they have no theoretical justification. The Weibull distribution was successfully applied in a number of works for fitting experimental PEF inactivation data for a surviving fraction $S(t, E)$ [20, 21, 22, 23, 24]

$$S(t, E) = \exp(-(t/\tau(E))^{n(E)}). \tag{1}$$

Here, $\tau(E)$ and $n(E)$ are the time and shape parameters accounting for the effective inactivation time and survival curve concavity, respectively [25]. But the Weibull model is also empirical and physical meaning of the obtained parameters $\tau(E)$ and $n(E)$ was not elucidated yet. Lebovka and Vorobiev [26] proposed a theoretical model for description of the surviving curves of spherically-shaped bacteria with the cell size distribution. It was shown that the Weibull model can be successfully applied for fitting of the surviving curves during PEF treatment.

Microbial cells display a variety of shapes and dimensions [27, 28] depending on the culture condition and age. Size of cells varies between 0.1 μm and 10 μm. Generally, the following different shapes can occur: near-spherical, ellipsoidal or ovoid (cocci), cylindroidal (bacilli), and spiral or comma-like (spirilli). For example, cells of *Escherichia coli* and *Salmonella typhi* are rod-like and have 0.4-0.6 μm in diameter and 2 - 4 μm in length, cells of *Leptospira spp.* are very long rod with 0.1 μm in diameter and 20 μm in length, cells of *Staphylococcus spp* are spherical cells with diameter of

0.5-1.5 μm, *Sacchoromyces cerevisiae* have ellipsoid cells with the principal dimensions of 2-8 μm and 3-15 μm, respectively, cells of *Klebsiella pneumoniae* are ovoid with a mean dimension 0.4 μm and *Vibrio cholerae* have comma-like cells with the principal dimensions of 0.5 μm and 1.5-3 μm, respectively [27, 28].

In general case, the surviving kinetics may be rather complex. An electroporation could be influenced by the aggregation of cells, their arrangement, local cell density, local solute concentration, and distribution of local electric field [29, 30, 31, 32]. The killing probability of non-spherical cells depends substantially on spatial orientation and changes from cell to cell [9, 33, 34] and can be related with cell diameters, spatial and orientational distributions.

In this work, a theoretical model allowing to describe the survivor curves for disordered or partially oriented non-spherical bacteria is formulated. The model predict how the cell orientation influences the lifetime of a spheroidal microbial cell exposed by PEF. The numerical simulations of surviving kinetics of disordered and partially ordered suspensions of microbial cells were done.

2. Description of the model

2.1. Transmembrane potential

In general case, electroporation consists of different stages including the charging of the membrane, creation of pores and evolution of pore radii [35]. For a single spherical cell under the steady state conditions, the transmembrane potential depends on the angle φ between the external field E direction and the radius-vector r on the membrane surface [36]:

$$u_m = 1.5fRE\cos\varphi. \tag{2}$$

Here, R is the cell diameter, and f is a parameter depending on electrophysical and dimensional properties of the membrane, cell and surrounding media. In dilute suspensions of cells, the value of f is close to 1.

The value of u_m is proportional to the cell radius R. The highest drop of potential occurs at the cell poles and decreases to zero at $\varphi = \pm\pi/2$. So, the larger microbial cells get killed before smaller ones and the damage probability is maximal at the cell poles.

If a cell is non-spherical, the transmembrane potential u_m becomes more complex function of the cell size and geometry, direction of external field and position on the membrane surface. The transmembrane potential u_m of an arbitrary oriented ellipsoidal cell at some point on the membrane surface $r(x, y, z)$ may be calculated from the following generalized Schwan equation [38]:

$$u_m = \sum_{i=x,y,z} r_i E_i/(1 - L_i). \tag{3}$$

Here, L_i are the depolarising factors defined by the cell radii R_x, R_y and R_z [39]. This approximation works for a membrane with negligibly small conductance and its application was discussed extensively in literature [34, 40, 41, 42, 43, 44].

The depolarizing factor of a prolate spheroid ($R_z > R_x = R_y$) along the symmetry axis z is [39]

$$L_z = \frac{1-e^2}{2e^3}(\ln\frac{1+e}{1-e} - 2e), \quad e = \sqrt{1-a^{-2}}, \tag{4}$$

and for an oblate spheroid ($R_z < R_x = R_y$) it makes

$$L_z = \frac{1}{e^3}(e - \sqrt{1-e^2}\arcsin e), \quad e = \sqrt{1-a^2}, \tag{5}$$

where $a = R_z/R_x$ is an aspect ratio (major / minor axis). The depolarizing factors in x and y directions are defined as

$$L_x = L_y = (1 - L_z)/2. \tag{6}$$

Here, $L_x = L_y = L_z = 1/3$ for a spherical cell, $L_x = L_y \approx 0.5$, $L_z \approx 0$ for a long cylinder and $L_x = L_y \approx 0$, $L_z \approx 1$ for a thin disk.

In general case, the transmembrane potential can be calculated from (3), but a simpler form of this equation may be considered due to the symmetry of spheroid [34]. When the electric field vector lies in a $X0Z$ plane (see figure 1), (3) can be rewritten as

$$u_m = xE\sin\theta/(1-L_x) + zE\cos\theta/(1-L_z), \tag{7}$$

where θ is an angle between the external field and symmetry axis of spheroid, and x and z are coordinates of a point at the spheroid surface. The values of x and z in a spheroidal system are defined as [45]

$$x = R_x\sqrt{1-\eta^2}\cos\phi, z = R_z\eta, \tag{8}$$

where $-1 \le \eta \le 1$, $0 \le \phi \le 2\pi$ are the spheroidal coordinates.

Finally, introducing (8) into (7), we obtain

$$u_m = ERa^{-1/3}(\frac{\sin\theta\cos\phi\sqrt{1-\eta^2}}{1-L_x} + \frac{\cos\theta a\eta}{1-L_z}), \tag{9}$$

where R is a radius of sphere, that has the same volume V as spheroid ($V = 4\pi R^3/3 = 4\pi R_z R_x^2/3$).

2.2. Lifetime of a microbial cell exposed by PEF

The lifetime of a membrane in some point at the spheroid surface can be estimated on the basis of the transient aqueous pore model [14]:

$$\tau(u_m(\theta,\eta,\phi)) = \tau_\infty \exp\frac{\pi\omega^2/kT\gamma}{1+(u_m(\theta,\eta,\phi)/u_o)^2}. \tag{10}$$

Here, τ_∞ is the parameter ($\tau \to \tau_\infty$ in the limit of very high electric fields), ω and γ are the line and surface tensions of a membrane, respectively, $k = 1.381{\cdot}10^{-23}$ J/K is the Boltzmann constant, T is the absolute temperature, $u_o = \sqrt{2\gamma/(C_m(\varepsilon_w/\varepsilon_m - 1))}$ is the voltage parameter (the dimension of u_o is Volts), C_m is the specific capacitance of a membrane, $\varepsilon_w, \varepsilon_m$ are the relative dielectric permittivities of the aqueous phase and of the membrane, respectively.

The lifetime of a spheroidal cell τ_c depends on the angle θ between electric field direction E and the symmetry axis Z of a spheroid. It can be estimated by a averaging of $\tau^{-1}(u_m)$ on the spheroid surface:

$$\tau_c^{-1}(\theta) = R_x^2 A^{-1} \int\limits_{-1}^{1} \int\limits_{0}^{2\pi} \frac{\sqrt{a^2(1-\eta^2)+\eta^2}}{\tau(u_m(\theta,\eta,\phi))} d\eta d\phi, \tag{11}$$

where

$$A = 2\pi R_x^2 \int\limits_{-1}^{1} \sqrt{a^2(1-\eta^2)+\eta^2} d\eta \tag{12}$$

is the surface area of a spheroid [45].

For a prolate spheroid, the surface area is

$$A = 2\pi R_x^2 (1 + \frac{a \arcsin \sqrt{1-a^{-2}}}{\sqrt{1-a^{-2}}}), \tag{13}$$

and for a oblate spheroid it makes

$$A = 2\pi R_x^2 (1 + \frac{a \arcsin h\sqrt{a^{-2}-1}}{\sqrt{a^{-2}-1}}). \tag{14}$$

2.3. Surviving probability during a PEF treatment

A surviving probability of a single spheroid with the angle θ of its principal axis relative to the external electric field E is defined as

$$S(t,\theta) = \exp(-t/\tau_c(\theta)). \tag{15}$$

Then, a surviving probability $S(t)$ of the whole suspension with spheroids of different spatial orientation can be calculated as:

$$S(t) = \int\limits_{-1}^{1} f(\theta) \exp(-t/\tau_c(\theta)) d\cos\theta, \tag{16}$$

where $f(\theta)$ is an angular orientational distribution function of spheroids.

For randomly oriented spheroids $f(\theta) = 1/2$. For partially oriented spheroids it is useful to introduce an order parameter Q defined as [46]:

$$Q = \frac{1}{2} \int\limits_{-1}^{1} f(\theta)(3\cos^2\theta - 1) d\cos\theta. \tag{17}$$

For perfectly oriented suspension, when all spheroids are completely aligned, $Q = 1$ and for randomly oriented suspension the order parameter is zero, $Q = 0$.

Disordered suspensions of anisotropic cells may be oriented by the external electric or magnetic fields [47, 48, 49, 50], or by the fluid flow [51, 52, 9]. In the external electric field E the angular orientation distribution function $f(\theta)$ can be estimated as [53, 46]

$$f(\theta) = \frac{\exp(U^* \cos^2\theta) d\cos\theta}{\int\limits_{-1}^{1} \exp(U^* \cos^2\theta) d\cos\theta}, \tag{18}$$

where U^* is a dimensionlees electrostatic energy of spheroid in the external field E,

$$U^* = \beta E^2/(2kT). \tag{19}$$

Here, β is the electrical polarizability anisotropy of a particle that depends on electrophysical properties of the particle and the outer solution.

An order parameter Q can be calculated by substitution of (18) into (17) [54]

$$Q = \frac{3}{4\sqrt{U^*}}(\exp(U^*)/\int\limits_0^{\sqrt{U^*}} \exp(t^2)dt - 1/\sqrt{U^*}) - 1/2. \tag{20}$$

Proceeding from (16)-(20), surviving kinetics versus order parameter Q can be calculated for partially ordered microbial cells.

2.4. Details of numerical calculations

For $\tau_c(\theta)$ evaluation using (9)-(12), the double integration was done using Simpson's quadrature rule. The accuracy of numerical integration was better than 10^{-6}. The voltage scale parameter in (10) was estimated as $u_o \approx 0.17$V from data obtained by Lebedeva [55] for the general lipid membranes ($\omega \approx 1.69 * 10^{-11}$N, $\gamma \approx 2 * 10^{-3}$ N/m, $\varepsilon_w \approx 80$, $\varepsilon_m \approx 2$, $C_m \approx 3.5*10^{-3}$F/m^2 at $T = 298$K). The time scale parameter was put as $\tau_\infty \approx 3.7 * 10^{-7}$s [55]. It is useful to use in calculations a dimensionless reduced field intensity defined as $E^* = E/E_o$, where $E_o = 2u_o/3R$, and R is an equivolume radius of a spheroid. Note that at $2R \approx 1\mu$m, and $u_o \approx 0.17$V, $E_o \approx 2.27$kV/cm. All these parameters were used calculation for estimation purposes.

The calculated dependencies of $\tau_c(\theta)$ were used for numerical calculation of the surviving kinetics from (16) at different values of order parameters Q ((20)).

3. Results and discussion

3.1. Lifetime of a spheroidal microbial cell

Figure 2 and figure 3 present some examples of the calculated relative lifetime τ_c/τ_∞ versus reduced field intensity E^* for a prolate (2) and oblate (3) spheroids at different values of angle θ. A prolate spheroid in external electric field was more stable at $\theta = 90^o$ and less stable at $\theta = 0^o$ than a spherical cell of the same volume, but an oblate spheroid was always less stable electrically than a spherical cell of the same volume.

The relative lifetime τ_c/τ_∞ versus angle θ for different aspect ratio a for a prolate and oblate spheroids at E^*=10 are presented in figure 4 and figure 5.

For a prolate spheroid, the value of τ_c/τ_∞ considerably increases with angle θ increase and it was a minimum for a cell aligned along the applied field E (figure 4). This result is in accordance with experimental observations of [34], who reported minimum electropermeabilization for the cells aligned along to the electric field direction.

For an oblate spheroid, the value of τ_c/τ_∞ was smaller than for a spherical cell of the same volume and the value of τ_c/τ_∞ decreases with angle θ increase (figure 5). It

was maximal for $\theta = 0$, but τ_c/τ_∞ dependence versus angle θ was not so distinct as for a prolate spheroid.

There exist some threshold angle $\theta = \theta_t$, at which the curve $\tau_c/\tau_\infty(E^*)$ for a prolate spheroid is very close to that for a spherical cell of same volume. The cell permeabilization was suppressed at $\theta > \theta_t$ and at was enhanced $\theta < \theta_t$ as compared with a spherical cell of the same volume. The higher was the aspect ratio a, the larger was the threshold angle θ_t. For an oblate spheroid, the threshold angle $\theta = \theta_t$ was observed only at high aspect ratio $a > 0.3$.

3.2. Survivor kinetics of a disordered suspension of cells

Due to the Brownian motion a random orientational distribution for suspensions of microbial cells is typical when field-induced ordering effects are absent. Figure 6 and figure 7 show the calculated survivor curves $S(t)$ of disordered suspensions ($f(\theta) = 1/2$) in (16)). The ideal first order kinetics law (15) was observed for suspensions of identical spherical cells (dashed lines in figure 6 and figure 7). The deviations from first order kinetics for prolate spheroidal cells became more pronounced with decreasing of the electric field intensity E^* (figure 6) or increasing of the aspect ratio a (figure 7). The τ_c/τ_∞ versus θ dependence was not so pronounced for oblate cells as for prolate cells, and no noticeable deviations from the first order kinetics were observed.

The kinetics $S(t)$ demonstrates that surviving probability in disordered suspension was higher for prolate cells and was lower for oblate cells as compared with the surviving probability for spherical cells of equivalent volume (figure 7).

The calculated survivor curves $S(t)$ for the prolate cells may be fitted with empirical Weibull function (1). This model always gives only upward concavity, i.e. $n < 1$, for orientationally disordered suspensions of prolate cells. But the numerically estimated shape n and relative time τ/τ_∞ parameters were rather sensitive to the upper cutting boundary t_{max}/τ_∞. This fact reflects existence of an intrinsic inconsistency between an unknown survival function and Weibull function [26].

3.3. Survivor kinetics of a partially oriented suspension of cells

Figure 8 and figure 9 show the calculated survivor curves $S(t)$ for partially ordered suspensions of spheroidal cells. The surviving kinetics of more disordered suspensions ($Q \rightarrow 0$) in the limit of large time ($t/\tau_\infty \gg 1$) was obviously controlled by the inactivation of cells oriented perpendicular to the applied field, and $S(t) \approx \exp(-t/\tau(90^o))$.

Increase of the order parameter Q results in two different regimes of surviving kinetics that correspond to inactivation of cells oriented along the field (fast regime at small time $S(t) \approx \exp(t/\tau(0^o))$ and perpendicular to the applied field (slow regime at large time $S(t) \approx \exp(t/\tau(90^o))$. The partial contribution of the first (fast) regime to inactivation kinetics increases with growth of the order parameter Q (figure 8).

For partially orientationally ordered suspensions with given Q, increase of the electric field strength E^* causes enhancement of inactivation kinetics and two regimes of surviving kinetics are also observed (figure 8). The rate of inactivation in the regime of slow inactivation is not constant in the limit of large time ($t/\tau_\infty \gg 1$) and increases with E^* increase.

The orientational ordering can be induced during the PEF treatment. Because of the quadratic dependence (see equations (18),(19)) on the electric field strength E, the orientational ordering in high pulsed electric fields may be noticeable. The possibility of ordering for ellipsoidal or cylindrically shaped microorganisms under the effect of external electric fields is discussed in [56]. A rod-shaped tobacco mosaic virus (TMV, about 0.018 μm in diameter and 0.3 μm in length [57]) demonstrates a strong orientation, near to complete saturation of the optical birefringence, in the electric field as high as ≈4 kV/cm[54]. Electrooptical studies of rod-shaped *E. coli* suspensions [58, 59] shows the existance of strong orientational ordering at electric fields of $E < 1$ kV/cm.

The orientation electric field induced effects can be roughly estimated using (19),(20). Taking the experimental value of the electrical polarizability anisotropy of *E. coli* cells $\beta = 4{\cdot}10^{-27}$ Fm^2[58], we obtain from (19) that $U^* = \beta E^2/8\pi kT \approx 5{\cdot}10^3$ at $E = 1$ kV/cm and $T = 298$ K. The corresponding order parameter following from (20) is $Q \approx 1$, e.i., degree of ordering is high.

But the degree of orientation can depend also on the pulse duration. As it is shown schematically in figure 10, the order parameter Q increases with time constant τ_o after the external electric field is switched on. The order parameter Q decreases to zero with another time constant τ_x when the external field is switched off. The relaxation time τ_x is determined by the Brownian rotation diffusion of the spheroid rotation about x axis in absence of electric field.

According to [60], the rotational diffusion times of a spheroid with respect to the symmetry axes x (or y), and z are:

$$\tau_{x,y} = \frac{2(a^4 - 1)}{3a((2a^2 - 1)F - a)}\tau_R, \tag{21}$$

$$\tau_z = \frac{2(a^2 - 1)}{3a(a - F)}\tau_R, \tag{22}$$

where $\tau_R = \pi\eta R^3/kT$ is the rotational diffusion time for a sphere of radius R with the same volume as a spheroid and

$$F = F_p = \ln(a + \sqrt{a^2 - 1})/\sqrt{a^2 - 1}, \tag{23}$$

$$F = F_o = \arctan(\sqrt{a^{-2} - 1})/\sqrt{1 - a^2}, \tag{24}$$

for a prolate and oblate spheroid, respectively.

Insert in figure 10 shows a rotation diffusion time τ_x (along the short axis x) of the prolate spheroid versus an aspect ratio a as calculated from (21), (23). For example, the rotational diffusion time is of the order of $\tau_x \approx$1s for a *E. coli* cell with equivolume

radius $R \approx 0.64\mu$m and aspect ratio $a \approx 2$ [58], but it can increase substantially with increase of the equivolume radius R or aspect ratio a.

It is more difficulty to calculate the time τ_o characterizing the process of ordering in the external electric field . The estimations shows [46]that $\tau_o \approx \tau_x$ in a low field, when, $Q < 0.1$. At a very high electric field, when $Q \to 1.0$, $\tau_o \approx \tau_x/(2U^*/15)$, where $U^* = \beta E^2/(8\pi kT)$ (equation (19)).

For rather small *E. coli* cells from the above estimations, we obtain $\tau_o \approx \tau_x/(2U^*/15) \approx 10^{-3}$s at $E_o = 1$ kV/cm and $\tau_o \approx 10^{-5}$s at $E = 10$ kV/cm ($T = 298$ K). So, small cells like those of *E. coli* may be effectively oriented during the pulse duration at PEF treatment with field strength $E = 10$ kV/cm and pulse duration $t_i = 10^{-5}$s. This estimation is in accordance with experimental observations [62] showing that the 24 ms pulse causes the observable orientation of the *E. coli* cells parallel to the field direction only at fields E exceeding 1.25 kV/cm.

However, the PEF-induced orientation effects can be supressed for larger cells or in presence of cells aggregation. The factor of bacterial aggregation is essential, because particles of the large colloidal aggregates are bounded and can not be freely reoriented during the PEF treatment. The aggregation and colony formation is a typical phenomenon in biocolloidal suspensions of bacterial particles, and some kind of bacteria (e.g. *Bacillus subtilis*) exhibit various aggregation patterns [63, 64]. Moreover, the PEF-induced cell-cell aggregation are also inportant [65].

For larger cells, for example, with equivolume radius R of $\approx 5.0\mu$m the rotation diffusion time is $\tau_x \approx 10^2 - 10^3$ s (insert in figure 10). In this case $\tau_o \approx \tau_x/(2U^*/15) \approx 10^{-2} - 10^{-3}$ s at $E = 10$ kV/cm and $t_i \ll \tau_o$. So, PEF-induced orientational effects can be rather small and not contribute into inactivation kinetics for large cells or cell aggregates.

4. Conclusion and outlook

This study has demonstrated the possibility of non-exponential survaving kinetics of microbial inactivation under the PEF treatment, which is believed to be related to the orientational disorder existing in a suspension of spheroidal microbial cells. Another factor can be related with sizes distribution of microbial cells [26]. Deviations from the ideal first order kinetics law ((15)) are more pronounced in completely disordered suspensions of prolate spheroids at small electric field strength or at large aspect ratio a. Efficiency of inactivation enhances with order parameter and field strength increase in partially oriented suspensions. In general case the inactivation kinetics can be influenced by the concentration of cells, their aggregation and arrangement [30, 31, 32]. The relevance and importance of such effects for explanation of the survival curves observed in PEF-inactivation experiments should be studied in future. The accurate description of inactivation kinetics requires accounting for the dynamics of bacterial cell reorientation in a high electric field during its inactivation. It seems to be important also to find correlations between factors that controls aggregations of bacterial cells, PEF protocols

and parameters of bacterial inactivation kinetics.

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Figure legends

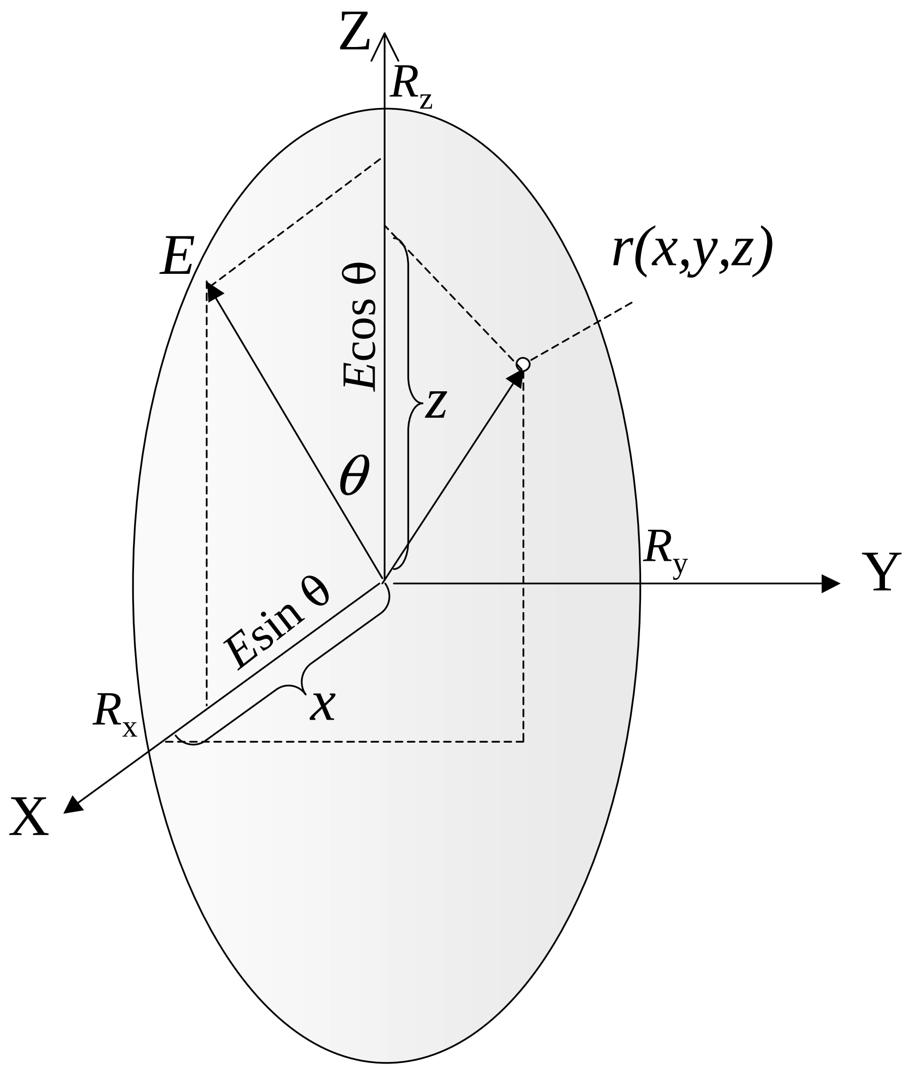

Figure 1. Schematic representation of the problem under consideration. X, Y, Z are the local Cartesian coordinates related to the oblate spheroidal microbial cells in the external field E (it is supposed here that it is lies in the XOZ plane). Here, θ is an angle between the electric field direction E and symmetry axis of spheroid Z, $r(x, y, z)$ is the radius of a membrane surface point, where the transmembrane potential is calculated, $R_x = R_y, R_z$ are the cell radii.

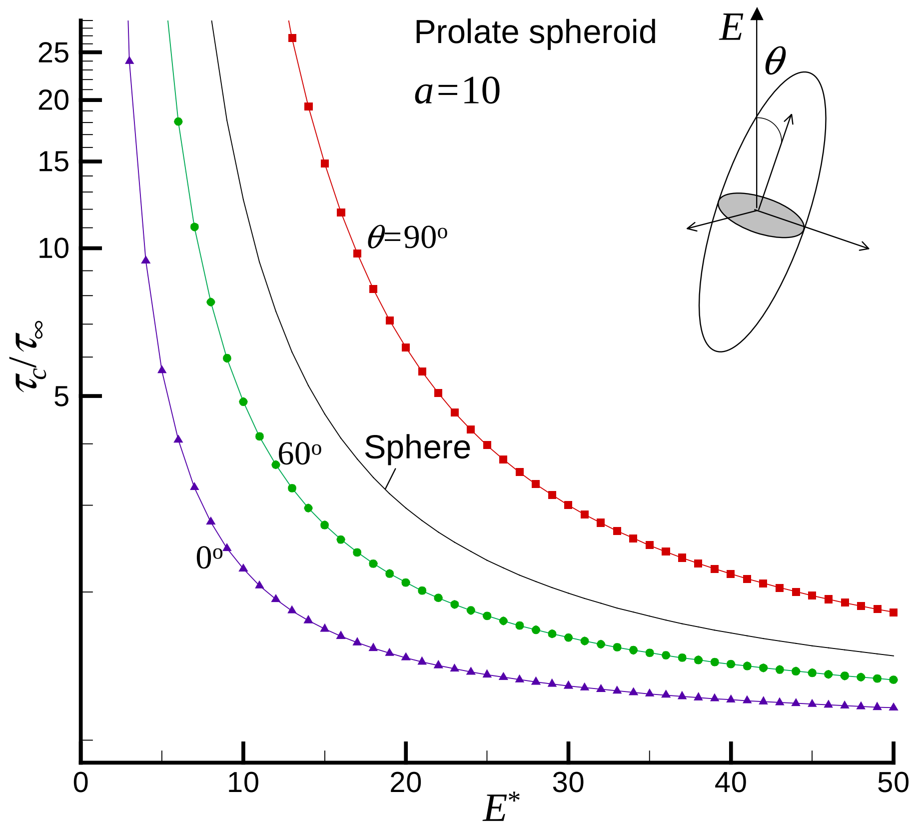

Figure 2. Relative lifetime τ_c/τ_∞ of a prolate (aspect ratio $a = 10$) cells versus reduced field intensity E^* at different angles θ between electric field direction and symmetry axis of spheroids. The solid lines show data for a spherical cell with a radius equivalent to that of an equivolume spheroid.

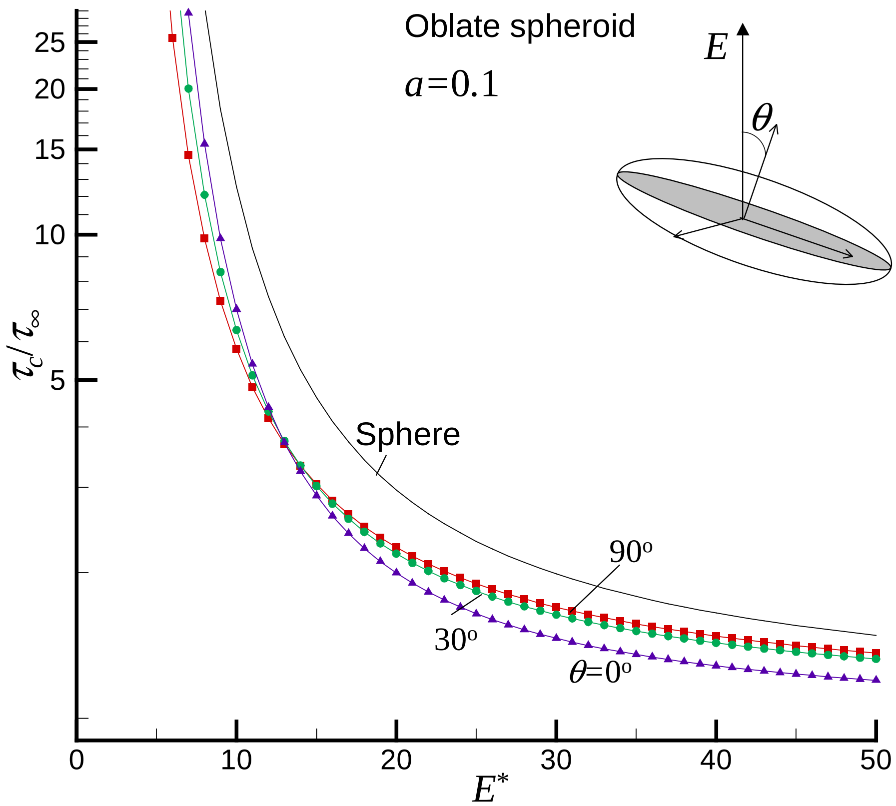

Figure 3. Relative lifetime τ_c/τ_∞ of a oblate (aspect ratio $a = 0.10$) cells versus reduced field intensity E^* at different angles θ between electric field direction and symmetry axis of spheroids. The solid lines show data for a spherical cell with a radius equivalent to that of an equivolume spheroid.

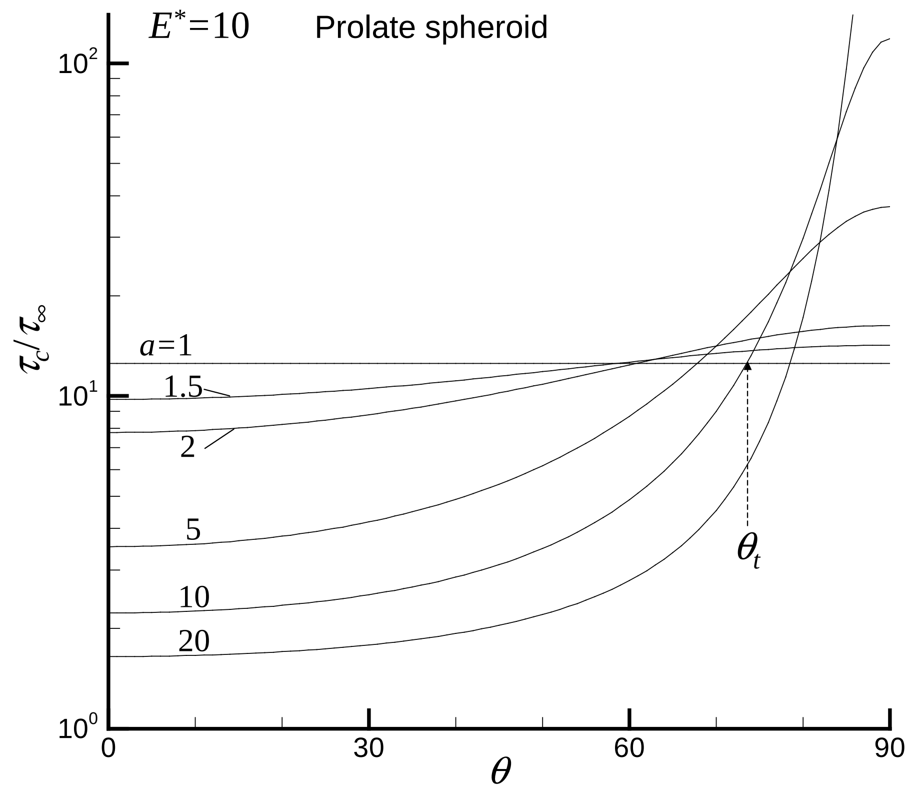

Figure 4. Relative lifetime τ_c/τ_∞ of a prolate spheroidal cell versus angle θ for different aspect ratio a. The calculation were done at the given value of reduced electric field intensity $E^* = 10$ that corresponds to $E \approx 22.7$ kV/cm for an equivolume radius of spheroid $2R \approx 1\mu$m, and voltage parameter $u_o \approx 0.17$V [55]. Arrows show threshold angles θ_t.

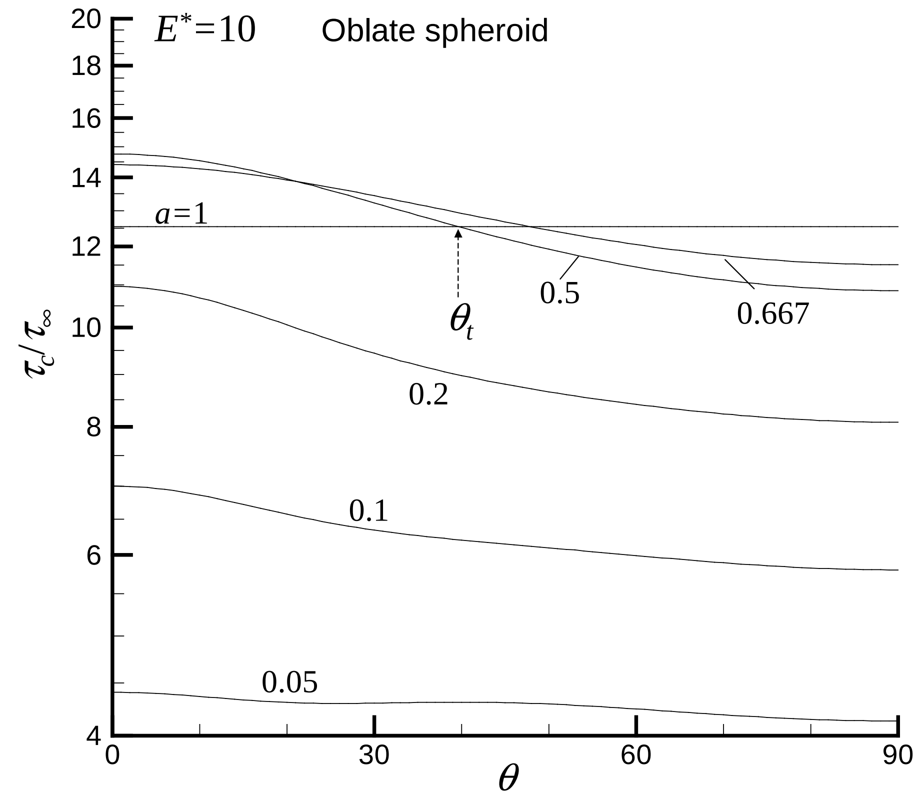

Figure 5. Relative lifetime τ_c/τ_∞ of a oblate spheroidal cell versus angle θ for different aspect ratio a. The calculation were done at the same condition as for data in figure 4. Arrows show threshold angles θ_t.

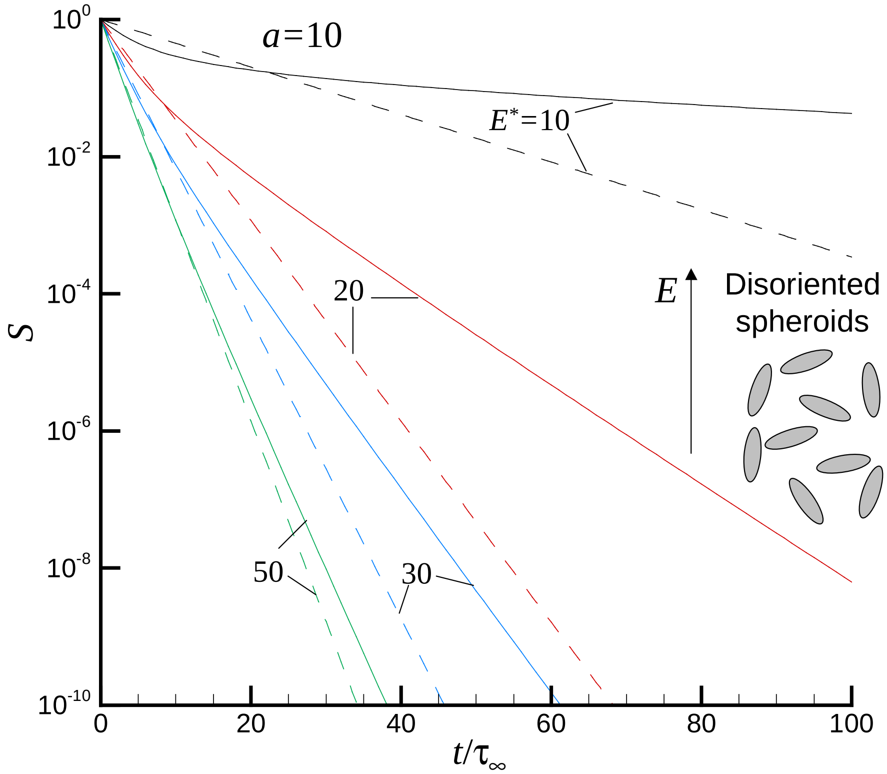

Figure 6. Survivor curves $S(t/\tau_\infty)$ for orientationally disordered spheroids at given $a = 10$ and different E^*. The dashed lines show data for spherical cell equivolume with spheroids.

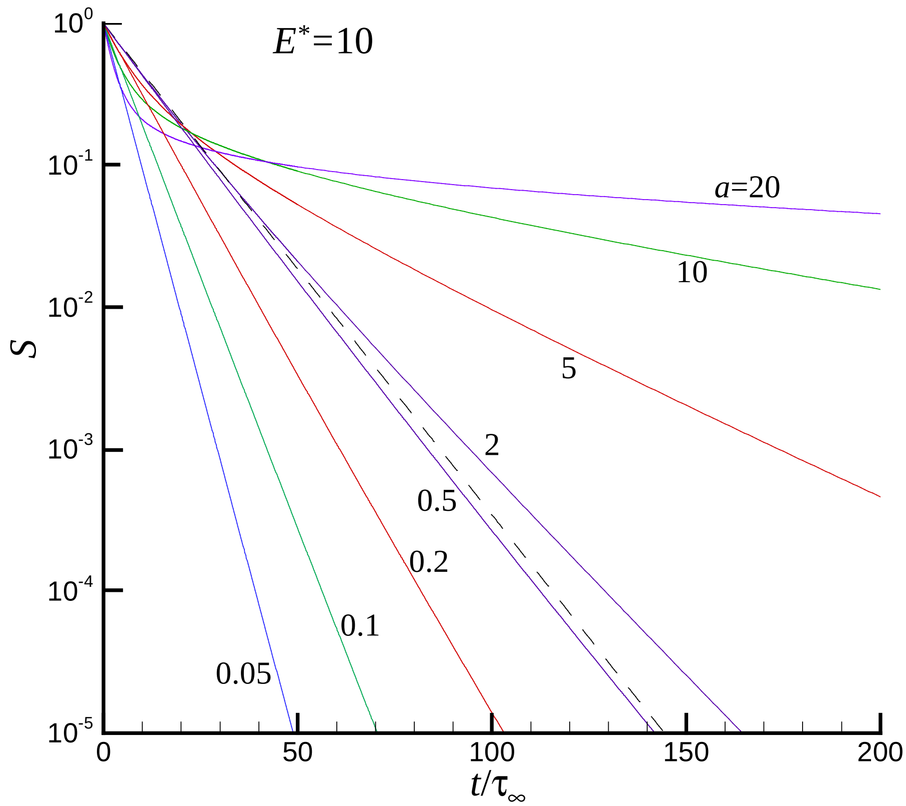

Figure 7. Survivor curves $S(t/\tau_\infty)$ for orientationally disordered spheroids at given E^*=10 and different a . The dashed lines show data for spherical cell equivolume with spheroids.

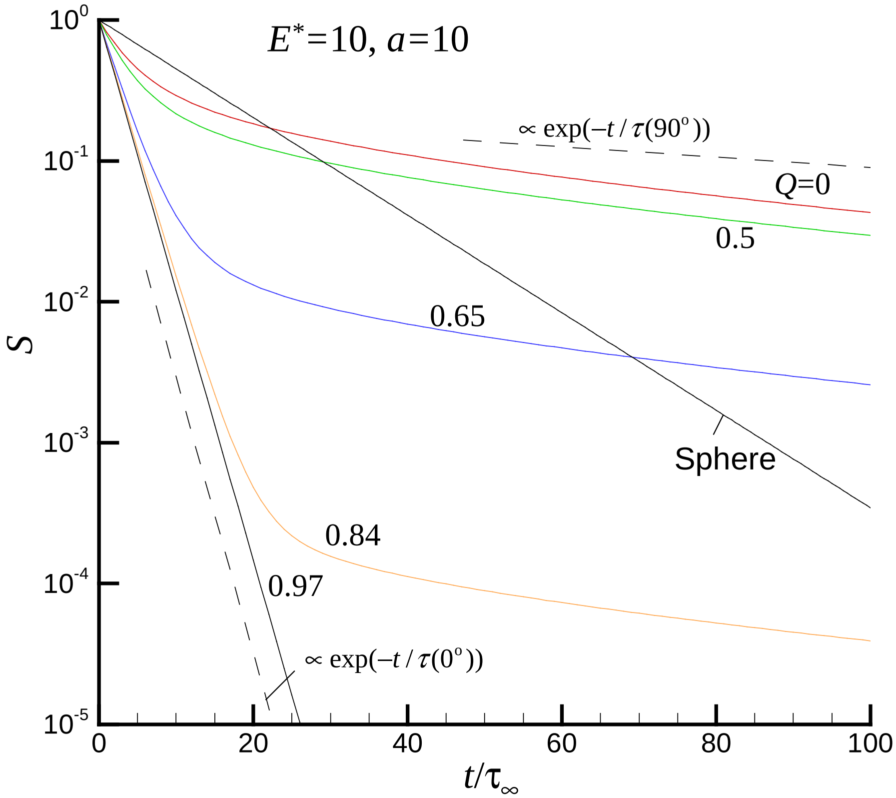

Figure 8. Survivor curves $S(t/\tau_\infty)$ for partially ordered suspensions of prolated spheroids at $a = 10$, $E^* = 10$ and different Q values. The dashed lines show the slopes that correspond to orientation with θ=0 and θ =90^o.

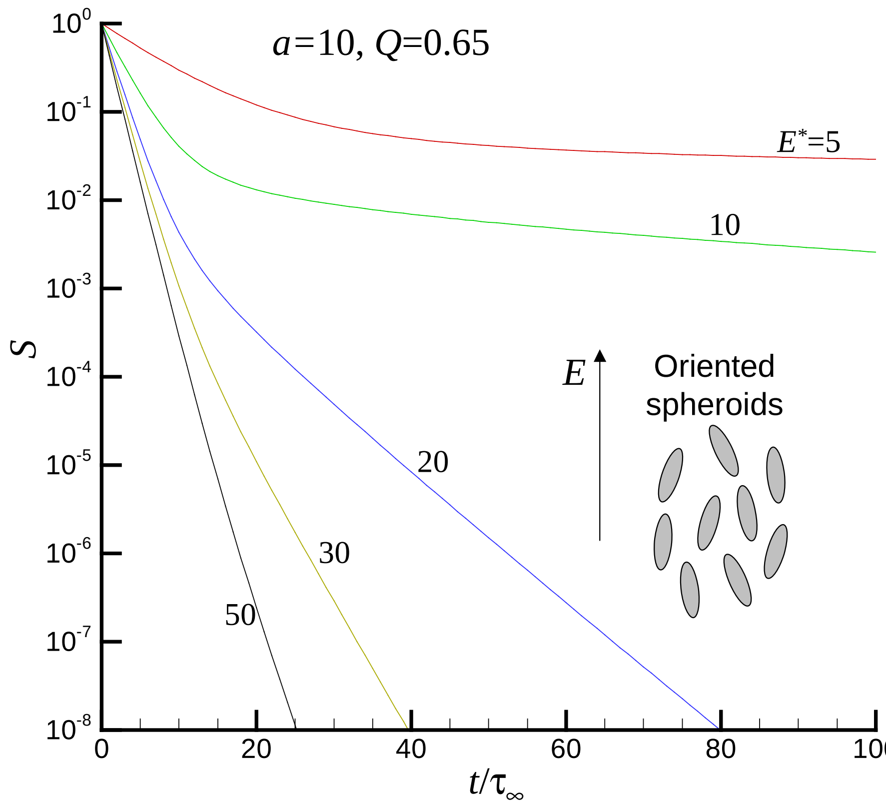

Figure 9. Survivor curves $S(t/\tau_\infty)$ for partially ordered suspensions of prolated spheroids at $a = 10$, $Q = 0.65$ and different E^* values . The dashed lines show the slopes that correspond to orientation with θ=0 and θ =90^o.

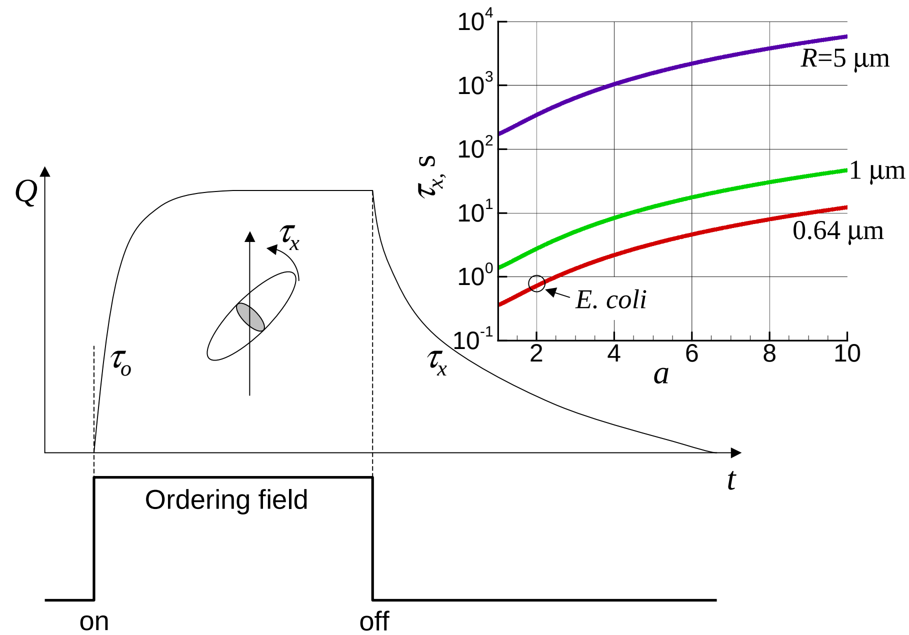

Figure 10. Changes of order parameter Q versus time t in the external electric field. Here, τ_x is the diffusion time for spheroid rotation about x axis, τ_o is the same diffusion time in the presence of the external field. Insert shows τ_x versus aspect ratio a for prolate spheroids, estimated from (21) and (23), $T = 298$ K, $\eta = 8.91{\cdot}10^{-4}$ Pa·s (water viscosity [61]), R is the radius of a sphere with the same volume as spheroid.